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# Anthropogenic water bodies as drought refuge for aquatic macroinvertebrates and macrophytes



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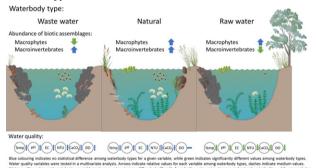
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#### HIGHLIGHTS

- As disturbance becomes more frequent or severe, habitat for aquatic biota declines.
- Aquatic biota in waste and raw-water storages was compared to natural water bodies.
- Macroinvertebrates in waste-water storages were comparable to natural water bodies.
- Macrophytes in raw-water storages were comparable to natural water bodies
- Anthropogenic storages could potentially offer important refuge during disturbance.

#### GRAPHICAL ABSTRACT

Blue colouring indicates no statistical difference among waterbody types for a given variable, while green indicates significantly different values among waterbody types. Water quality variables were tested in a multivariate analysis. Arrows indicate relative values for each variable among waterbody types, dashes indicate medium values. Water quality and macroinvertebrate assemblages in raw-water storages differed significantly from both waste-water storages and natural waterbodies, while macrophytes in waste-water storages differed from the other two types.



## ARTICLE INFO

Article history:
Received 11 August 2017
Received in revised form 31 October 2017
Accepted 31 October 2017
Available online 10 November 2017

Editor: D. Barcelo

Keywords:
Refugia
Man-made waterbodies
Pond
Lake
Wastewater treatment pond
Potable water reservoir

## ABSTRACT

Ecological research associated with the importance of refuges has tended to focus on natural rather than anthropogenic water bodies. The frequency of disturbances, including drought events, is predicted to increase in many regions worldwide due to human-induced climate change. More frequent disturbance will affect freshwater ecosystems by altering hydrologic regimes, water chemistry, available habitat and assemblage structure. Under this scenario, many aquatic biota are likely to rely on permanent water bodies as refuge, including anthropogenic water bodies. Here, macroinvertebrate and macrophyte assemblages from waste-water treatment and rawwater storages (i.e. untreated potable water) were compared with nearby natural water bodies during autumn and winter 2013. We expected macroinvertebrate and macrophyte assemblages in raw-water storages to be representative of natural water bodies, while waste-water treatment storages would not, due to degraded water quality. However, water quality in natural water bodies differed from raw-water storages but was similar to waste-water treatment storages. Macroinvertebrate patterns matched those of water quality, with no differences occurring between natural water bodies and waste-water treatment storages, but assemblages in raw-water storages were attributable to low abundances of several taxa. Macrophyte assemblages in raw-water storages were representative of natural water bodies, but were less diverse and abundant in, or absent from, waste-water

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treatment storages. No clear correlations existed between any habitat variables and macroinvertebrate assemblages but a significant correlation between macrophyte assemblages and habitat characteristics existed. Thus, there were similarities in both water quality and macroinvertebrate assemblages between natural water bodies and waste-water treatment storages, and similarities in macrophyte assemblages between raw-water storages and natural water bodies. These similarities illustrate that anthropogenic water storages support representative populations of some aquatic biota across the landscape, and thus, may provide important refuge following disturbance where dispersal capabilities allow.

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#### 1. Introduction

The conservation of freshwater biodiversity has recently received much attention in aquatic ecology (Dudgeon et al., 2006). Freshwater ecosystems are highly vulnerable and the proportion of species at risk of extinction compared with terrestrial and marine ecosystems is exceptionally high (Strayer and Dudgeon, 2010; Collen et al., 2014). Further, freshwater habitats are disproportionately speciose relative to marine and terrestrial ecosystems, given that fresh waters cover 0.8% of the earth's surface, yet support 6% of all described species (Dudgeon et al., 2006). Declines in freshwater biodiversity have been associated with many anthropogenic drivers, but particularly climate-induced changes in hydrological regimes (Heino et al., 2009). For example, altered hydrological regimes are likely to interfere with the completion of aquatic life cycles, placing species at greater risk of local extinction (Bond et al., 2008). Thus, measures to enhance the likelihood of maintaining biodiversity are particularly important in freshwater ecosystems.

Physical disturbances occur in all aquatic ecosystems and are influential in structuring aquatic communities (Boix et al., 2010). Disturbances affecting freshwater biota can be either biotic (e.g. predation) or abiotic (e.g. drought), and natural (e.g. flood) or anthropogenic (e.g. chemical spill) (Magoulick and Kobza, 2003). Drought, like many other disturbances, is a natural feature of many aquatic ecosystems worldwide (Humphries and Baldwin, 2003) and is a recurrent phenomenon in Australia (Bond et al., 2008; Lake, 2011). However, the frequency and severity of drought events are predicted to increase under climate change (Steffen and Hughes, 2013), negatively affecting freshwater ecosystems, particularly lentic systems, due to reduced runoff and increased drying (Bond et al., 2008; Steffen and Hughes, 2013). The effects of drought occur at a regional scale, so have the potential to threaten not only individuals but entire populations of aquatic biota and biodiversity across relatively large spatial scales (Bond et al., 2008).

Strategies for surviving disturbance include physiological mechanisms such as desiccation-resistant propagules or aestivation, and behavioural mechanisms to access refuges. Examples of such behavioural mechanisms include burrowing to access moist sediment and groundwater or aerial relocation to permanent water sources (Robson et al., 2011). Refuges typically provide a physical space that is protected from disturbance and can act as a source of colonists for the surrounding landscape after the disturbance passes (Sedell et al., 1990). Refuges that retain permanent surface water during drought are essential for the maintenance of most aquatic populations within a landscape (Humphries and Baldwin, 2003). However, the presence of numerous refuges and refuge types within a landscape is important because a suitable refuge for one species may not be suitable for others with differing life history traits (Robson et al., 2011).

As the intensity of human demand and disturbance increases on aquatic ecosystems, freshwater biota may rely on aquatic habitats that are anthropogenic in origin to persist in a highly-modified landscape, particularly when the availability of natural refuges diminish (Chester and Robson, 2013). Several anthropogenic water bodies support biodiverse aquatic assemblages, including waste-water treatment storages, fire dams, urban ponds, golf course lakes, roadside ditches and agricultural ponds (Chester and Robson, 2013; Mackintosh et al., 2015).

Anthropogenic wetlands are often small, but can contribute disproportionally to aquatic biodiversity within a region and, as such, several authors have argued for greater management priority for these habitats to help protect aquatic biodiversity (Davies et al., 2008; Halliday et al., 2015; Hill et al., 2016).

The ability for anthropogenic water bodies to act as potential refuges for aquatic biota has rarely been tested. Brainwood and Burgin (2009) investigated macroinvertebrate assemblages in farm dams in New South Wales, Australia and compared them with natural water bodies within the same landscape. Macroinvertebrate assemblages within farm dams were comparable to those of nearby natural lakes and ponds, and thus had valuable potential as drought refuges. Furthermore, these authors suggested that farm dams could also enhance connectivity among habitats that had been fragmented due to agricultural practices (Brainwood and Burgin, 2009). Another study in south-east Ireland demonstrated that macroinvertebrate assemblages in wastewater treatment storages were comparable to surrounding natural water bodies, again suggesting that anthropogenic water bodies could provide an important source of refuge during drought (Becerra Jurado et al., 2009). Numerous navigation canals in Europe contain diverse macrophyte assemblages, often including rare species (Goulder, 2008; Dorotovičová, 2013) and thus provide another good example of aquatic biota utilising man-made water bodies. In a study complementary to ours, Halliday et al. (2015) found no significant differences in fish, zooplankton and frog assemblages among natural water bodies, waterwater treatment storages and raw-water storages (i.e. systems that store potable water prior to treatment) in south-west Victoria, Australia suggesting the utility of those storages as refuges for local fauna.

The primary aim of this study was to compare macroinvertebrate and macrophyte assemblages from waste-water treatment storages and raw-water storages with natural water bodies in south-west Victoria, Australia. Based on the definition of Sedell et al. (1990), we contend that, for a water body to constitute a refuge from drying, it must retain water when natural lakes are dry (i.e. protected from the disturbance) and support representative biota to act as a source of colonists. A secondary aim was to explore relationships among biotic assemblages, water quality and habitat variables to identify characteristics of anthropogenic water bodies that may facilitate their role as refuge habitat for macroinvertebrates during extended periods of drought. We expected that macroinvertebrate and macrophyte assemblages within rawwater storages would be representative of those within surrounding natural water bodies, whereas assemblages in waste-water treatment storages were expected to be depauperate due to relatively poor water quality.

## 2. Methods

#### 2.1. Study location

A total of 18 water bodies were sampled across three water body types: raw-water storages (RWS); waste-water treatment storages associated with water reclamation plants (WRP); and natural water bodies (NWB), situated in south-west Victoria, Australia (Fig. 1), as per Halliday et al. (2015). Raw-water storages and waste-water treatment storages were managed by Wannon Water, a water authority operating

across five catchments in south-west Victoria. All storages sampled were located in an agricultural landscape with the exception of Lake Hamilton, which was located on the outer edge of an urban landscape.

The RWS varied in their physical characteristics (Table 1). For example, Plantation Rd. Storage and Cobden Service Basin had similar characteristics, both were completely surrounded by steep, bluestone cobble walls and devoid of macrophytes. In contrast, Ewen's Hill, Tank Hill, Glenthompson and Hartwichs Reservoirs had variable physical and habitat characteristics. These RWS were characterised by a single bluestone cobble dam wall at one edge of each storage, wide shallow edges with variable sediment grain sizes surrounding the rest of the storage, emergent and submerged macrophytes and substantial riparian vegetation consisting of eucalypts (*Eucalyptus* spp.) and blackwoods (*Acacia* spp.). Water in each of the RWS was supplied from one of a number of different sources (Table 1).

Waste-water treatment storages, or water reclamation plants (WRP), are used for storing treated sewage. The arrangement of WRP can vary in number among locations, ranging from a single storage to multiple ponds. The WRP sampled were the last ponds in the water treatment process. Water from these storages is recycled, being used for industry, agricultural irrigation or recreational facility irrigation.

WRP consisted of banks that were comprised completely of blue-stone cobble, and also had no riparian vegetation (Table 1). Monivae Winter Storage was an exception by having a single bluestone cobble wall and was otherwise surrounded by grassland, but still had no

other riparian vegetation. Three WRP contained macrophyte assemblages, but not as abundant or diverse as those in the other storage types. Several WRP had nearby streams: Muddy Creek flows past Monivae Winter storage approximately 80 m away; Konongwootong Creek flows past Coleraine WRP approximately 50 m away; and the Glenelg River flows past Casterton WRP approximately 150 m away.

Criteria for selecting natural water bodies (NWB) included that they were comparable to the anthropogenic storages in size, distribution and were not regularly maintained or regulated. These criteria were difficult to meet due to the previous summer (2012 – 2013) being extremely dry, with Australia experiencing the hottest January on record and Victoria experiencing one of the driest summers on record (Steffen and Hughes, 2013). As a result, many NWB that met these criteria were either too dry to sample or were inaccessible (Appendix A). Consequently, this limited the number and availability of NWB and thus some NWB were larger than originally anticipated. Furthermore, two NWB (i.e. Lake Aringa and Lake Hamilton) were anthropogenic in origin, but water levels are no longer actively managed as per WRP and RWS storages, so were included as quasi-natural.

NWB had the most variable physical characteristics and habitat (Table 1). All NWB were populated with macrophytes and were surrounded by riparian vegetation, except for Deep Lake, which had few submerged macrophytes and little riparian vegetation. Deep Lake, Lake Hamilton and Yatmerone Reserve had retaining walls constructed of cobble and boulders to support steep banks. Unlike any of the

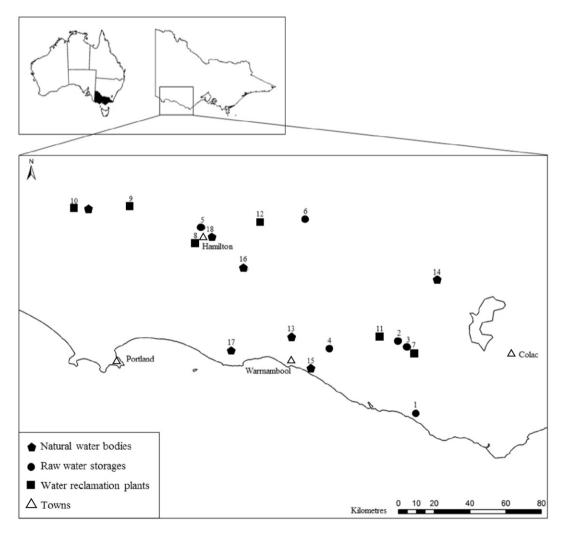


Fig. 1. Location of study water bodies in south-west Victoria, Australia. Numbers identify individual water bodies, corresponding with the 'Map no.' column in Table 1.

Table 1
Characteristics of each water body sampled including size, location, water source for the raw-water storages, effluent discharge regime of the water reclamation plants and river or creek connections for natural water bodies.

| Raw-water storages     |         |           |               | GPS coordinates (UTM) |              | TM)     |   |
|------------------------|---------|-----------|---------------|-----------------------|--------------|---------|---|
| Storage name           | Map no. | Size (ha) | Nearest town  | Zone                  | N            | Е       | Water sources   |
| Plantation Rd. Storage | 1       | 2.5       | Port Campbell | 54H                   | 0683644      | 5721802 | North Otway Pipeline  |
| Ewen's Hill Reservoir  | 2       | 14.2      | Cobden        | 54H                   | 0677337      | 5762139 | North Otway Pipeline  |
| Cobden Service Basin   | 3       | 1.7       | Cobden        | 54H                   | 0681127      | 5758777 | North Otway Pipeline  |
| Tank Hill Reservoir    | 4       | 11.6      | Framlingham   | 54H                   | 0646674      | 5758665 | North Otway Pipeline  |
| Hartwichs Reservoir    | 5       | 10.6      | Hamilton      | 54H                   | 0591585      | 5826865 | Grampians Supply System   |
| Glenthompson Reservoir | 6       | 3.1       | Glenthompson  | 54H                   | 0637099      | 5831189 | Yuppeckiar Creek Catchment  |
| Waste-water storages   |         |           |               | GPS coordinates (NTU) |              | TU)     |   |
| Storage name           | Map no. | Size (ha) | Nearest town  | Zone                  | N            | Е       | Effluent discharge  |
| Cobden WRP             | 7       | 3.6       | Cobden        | 54H                   | 0683373      | 5755676 | Reuse via irrigation  |
| Monivae Winter Storage | 8       | 16.2      | Hamilton      | 54H                   | 0588079      | 5818604 | Reuse via irrigation/industrial process waste                       |
| Coleraine WRP          | 9       | 2.2       | Coleraine     | 54H                   | 0559654      | 5838925 | Reuse via irrigation  |
| Casterton WRP          | 10      | 3.0       | Casterton     | 54H                   | 0535026      | 5837993 | Reuse via irrigation  |
| Terang WRP             | 11      | 10.2      | Terang        | 54H                   | 0668787      | 5764450 | Reuse via irrigation  |
| Dunkeld WRP            | 12      | 1.0       | Dunkeld       | 54H                   | 6173744      | 5829844 | Reuse via irrigation  |
| Natural water bodies   |         |           |               | GPS co                | ordinates (N | TU)     |   |
| Storage name           | Map no. | Size (ha) | Nearest town  | Zone                  | N            | E       | River/creek connection  |
| Lake Cartcarrong       | 13      | 24.1      | Winslow       | 54H                   | 0627420      | 5766033 | None  |
| Deep Lake              | 14      | 93.2      | Derrinallum   | 54H                   | 0690985      | 5799414 | Artificial drain to Lake Logan                                      |
| Lake Gillear           | 15      | 18.2      | Warrnambool   | 54H                   | 0639007      | 5745511 | Artificial drain to Hopkins Estuary                                 |
| Yatmerone Reserve      | 16      | 13.0      | Penshurst     | 54H                   | 0611698      | 5806818 | None  |
| Lake Aringa            | 17      | 16.0      | Port Fairy    | 54H                   | 0602472      | 5755862 | Artificial drain from surrounding swamps. Spills into Goose Lagoon. |
| Lake Hamilton          | 18      | 39.8      | Hamilton      | 54H                   | 0591645      | 5823357 | Grange Burn Creek   |

anthropogenic storages, there was direct stock access to some parts of Lake Cartcarrong, Deep Lake, Lake Gillear and Yatmerone Reserve. Lake Aringa and Yatmerone Reserve are both situated inside wildlife reserves, although approximately 2% of the total area of Yatmerone Reserve is accessible to livestock. All of the NWB are used for recreational fishing and boating. To facilitate recreational use, there are boat ramps and jetties at Deep Lake, Lake Aringa, Lake Hamilton and Lake Cartcarrong.

## 2.2. Study design

There were six replicates of each of the three water-storage types sampled (giving a total of 18 water bodies): RWS; WRP; and NWB. The surface area of all anthropogenic storages ranged from approximately 1 to 16 ha and NWB ranged from approximately 13 to 93 ha (Table 1).

Sampling was conducted on two occasions, the first during mid-autumn (4th–25th April) and the second in late-winter (24th–27th August) 2013. Storages were sampled in random order, with some constraints. RWS were not sampled directly after WRP due to concerns with potential contamination of the RWS. It was also impractical to completely randomise the order in which the storages were sampled due to the time needed to travel among them. Due to these constraints, storages were allocated to seven groups based on location and then the groups were randomly sampled. Three individual sites were sampled within each storage, with sites randomly located following selection of a haphazard starting point.

## 2.3. Water quality

Water quality variables including water temperature (°C), electrical conductivity ( $\mu$ S/cm), dissolved oxygen (mg/L), pH, alkalinity (mg/L) and turbidity (NTU) were measured at the water surface at three sites within each storage. Due to the availability of water quality instruments, it was necessary to use different instruments for each sampling occasion. During the first sampling occasion, water temperature, electrical conductivity, dissolved oxygen, and pH were measured using a YSI

Professional Plus handheld multiparameter meter (Yellow Spring, Ohio, USA) and turbidity was measured using a Hach 2100Q portable turbidity meter (Loveland, Colorado, USA). At Yatmerone Reserve on the first sampling occasion, water temperature, electrical conductivity, turbidity and dissolved oxygen were measured with a YEOKAL Intelligent Water Quality Analyser (model 611) (Brookvale, New South Wales, AUS) and pH was measured using a HANNA Instruments pH meter (model HI-98127) (Woonsocket, Rhode Island, USA). During the second sampling occasion, water temperature, electrical conductivity and pH were measured using a Hanna Instruments pH/conductivity meter (model HI-98129) (Woonsocket, Rhode Island, USA), dissolved oxygen was measured using a Hanna Instruments Portable Dissolved Oxygen Meter (model HI-9146) (Woonsocket, Rhode Island, USA) and turbidity (NTU) was measured using a Hach 2100Q portable turbidity meter (Loveland, Colorado, USA). Alkalinity was measured once at each water body on each sampling occasion using a Hach alkalinity test kit (model AL-TA) (Loveland, Colorado, USA). The percent coverage of shade was visually estimated at each site.

## 2.4. Substrate characteristics

Substrate grain size was estimated at each site within each storage using the Wentworth-Lane scale (Wentworth, 1922) by visually assessing the sample area along each 5-m transect used for macroinvertebrate sampling. The substrate was then placed into categories based on grain size and the percentage contribution that each sediment category made to the entire area was estimated.

## 2.5. Macrophyte sampling

Macrophyte coverage was measured at each site within each storage using a line-transect method. A 10-m transect was run perpendicular to the shoreline with the mid-point at the water's edge. Macrophytes that fell within 5 cm of either side of the transect were identified to the lowest possible taxonomic level (generally species) using dichotomous keys (Romanowski, 1998; Sainty and Jacobs, 2003) and the extent of each species along the transect was recorded.

#### 2.6. Macroinvertebrate sampling

An initial pilot survey found that habitat types varied considerably among storages, ranging from dam walls consisting entirely of bluestone cobble to banks that were surrounded by natural riparian vegetation and macrophytes. Due to this variation in habitat types, two sampling techniques, kick samples and rock scrubs, were used.

## 2.6.1. Kick samples

On each sampling occasion, one kick sample (Chessman, 1995) was taken from each of the three sites in each water body over 5 m to incorporate all habitat types (e.g. bare and vegetated substrata, macrophytes, large woody debris and rocks) using a dip net. Dip nets consisted of a  $320 \times 250$ -mm frame fitted with 250- $\mu$ m mesh. The three kick samples were combined to incorporate within-site variability for each storage.

#### 2.6.2. Rock scrub samples

Rock scrub samples were taken only on the first sampling occasion as preliminary analyses indicated that the taxa identified did not differ substantially to the kick samples. At each storage, 20 rocks of similar size were haphazardly chosen. Totally submerged rocks were selected preferentially, though this was not always possible, so partially submerged rocks were also included where necessary. Rocks were lifted directly into a net before being transferred to a bucket. Each rock was thoroughly scrubbed with a brush and then forceps used to remove any remaining macroinvertebrates. All 20 rocks were scrubbed into the same bucket, creating a single combined sample per storage. The longest and shortest dimensions of each rock were measured and used as a covariate in all statistical analyses, given that larger rocks might be expected to contain a greater number of invertebrates than smaller rocks. All samples (i.e. kick and rock scrub) were preserved in 70% ethanol for later identification in the laboratory.

#### 2.6.3. Laboratory methods

Some macroinvertebrate samples contained large amounts of organic detritus and large numbers of macroinvertebrates so were sub-sampled to a minimum of 12.5% using a Plankton Splitter (Folsom, 0.5 L). All macroinvertebrates within the sub-sample were sorted and counted and no single sub-sample contained fewer than 250 macroinvertebrates to ensure that all taxa were adequately represented. Furthermore, the remainder of the sample was scanned for a minimum of 5 min to ensure that any additional rare taxa were identified. Macroinvertebrates were identified to family level using dichotomous keys (Gooderham and Tsyrlin, 2009) with the exception of Lepidoptera (Order), Acarina (Order) and Ostracoda (Class). Chironomids were identified to subfamily. Family-level identification is regarded as sufficient to show patterns in macroinvertebrates assemblages on a large spatial scale (Marchant et al., 1995) and enabled a greater number of replicate water storages to be processed than possible using a finer taxonomic resolution. Sub-sample counts were mathematically adjusted for statistical analysis.

#### 2.7. Statistical analyses

Multivariate statistical analyses were performed in PRIMER v.6 with the PERmutational Multivariate ANalysis Of VAriance (PERMANOVA+) add-on. PERMANOVA is a permutation-based method for assessing significance and does not rely on the assumption of normality, which is regularly violated in ecology and thus can be more powerful than traditional ANOVA (Anderson et al., 2008). Sediment grain size, water quality variables and macrophyte assemblages were compared among storage types using a model consisting of three factors: storage type (fixed; 3 levels); storage nested within storage type (random; 18 levels); sampling occasion (random; 2 levels); with three replicates for each storage. Macroinvertebrate kick sample data were compared using a model consisting of two factors: sampling occasion (random; 2

levels); storage type (fixed; 3 levels) with six replicates for each storage type since replicate kick samples from each storage were pooled to form a single, representative sample. Rock scrub data were compared using a one-way PERMANCOVA with the factor storage type (fixed; 3 levels) and rock size as a covariate. Finally, all habitat variables (sediment grain size, water quality and macrophyte assemblages) were combined and compared among water storages using the same two-way PERMANOVA used for the macroinvertebrates kick samples.

The potential effect of differing water body sizes among storage types was tested using water body size as a covariate in the above multivariate analyses and a rarefaction was undertaken using EstimateS v.9.1.0 (Cowell, 2013) to adjust estimates of taxon richness for the water body surface area (Appendices B and C). No analysis detected a significant effect of (or interaction including) water body size, so the results presented hereafter do not include water body size.

All environmental data were normalised using mean standardisation in PRIMER v.7 and similarity matrices were calculated using Euclidean distance similarity measures. Transformations were necessary for macrophyte (square root) and both kick (log(x + 1)) and rock scrub samples (square root), with similarity matrices calculated using Bray-Curtis similarity measures including dummy variables of 1 where required. Non-metric multidimensional scaling (nMDS) plots provided visual patterns in these data, nMDS plots display samples as single points in 2-dimentional space, based on the relative similarity of assemblage diversity and abundances between each pair of samples (Clarke and Gorley, 2006). Shepard plots were used to determine whether the similarity measures used were appropriate for each data set. Pairwise comparisons were used to identify differences between storage types following significant PERMANOVA tests. Monte Carlo P values were reported in instances where there were insufficient unique permutations (<100) for a reliable test (Anderson et al., 2008). SIMilarity PERcentages (SIMPER) analysis were then used for both environmental and abundance data to identify which variable/taxa were influencing the dissimilarity between pairs of storages (Clarke and Gorley, 2006).

Separate RELATE analyses were used to assess whether macroinvertebrate assemblages and macrophyte assemblages were correlated with the combined habitat variables (Clarke and Gorley, 2006). This was followed by BEST analyses to further identify individual environmental variables (or combination of variables) that were correlated with the macroinvertebrate and macrophyte assemblages (Clarke and Gorley, 2006).

## 3. Results

## 3.1. Habitat and water quality variables

## 3.1.1. Comparison of sediment grain size among storage types

Clay/silt substrata were the most prevalent substrate occurring within all water storage types with cobble also common. There was a statistically-significant difference among storages nested within storage types (pseudo- $F_{15, 15} = 4.43$ , P = 0.001) and among storage types (pseudo- $F_{2, 13} = 2.77$ , P = 0.024; Table 2, Fig. 2a). Pairwise testing indicated that NWB and WRP differed from each other (t = 2.24, P = 0.003; Table 3), but neither differed from RWS. Cobble was more prevalent at WRP than NWB, while NWB had higher proportions of clay/silt substrate. Sediment grain size was consistent across sampling occasions.

## 3.1.2. Comparison of water quality among storage types

Average values of electrical conductivity, alkalinity, pH and turbidity were lower in RWS than WRP and NWB (Appendix D). Average levels of dissolved oxygen were similar among each storage type.

There was a significant sampling occasion by storage nested within storage type interaction for the water quality variables (pseudo- $F_{14,70}$  = 7.84, P = 0.001). There were also statistically-significant differences in water quality among storage types (pseudo- $F_{2,12}$  = 3.28, P =

0.001; Table 2, Fig. 2b). Water quality within RWS differed significantly from both WRP (t=2.53, P=0.001) and NWB (t=2.04, P=0.004; Table 3) but did not differ between WRP and NWB. SIMPER analysis failed to reveal any reliable water quality variables that explained differences among storage types, but qualitative trends suggested that RWS were clearer (i.e. had lower turbidity), had greater shade cover and lower conductivities and pH than the other two storage types (Appendix D).

## 3.2. Macrophytes

There were 17 macrophyte species identified across all storages (Appendix E). *Triglochin procerum* and *Juncus usitatus* were the two most commonly-occurring species, each found at seven sites. *Triglochin procerum* was the most abundant species across all the storages, with an average of 8% and 7% coverage during the first and second sampling occasions, respectively. Generally, there was lower recorded percent coverage of macrophytes during the second sampling occasion with four species: *Lepilaena australis*, *Ruppia* sp., *Elodea canadensis* and *Persicaria decipiens* not recorded at all. The second sampling occasion occurred shortly after substantial rainfall, which resulted in increased water levels at most sites. These macrophytes are generally submerged, so it is possible that they were still present, but in deeper water beyond our transects. No vegetation was recorded at the WRP at Cobden, Dunkeld and Monivae, nor at the RWS at Cobden and Plantation Rd.

Macrophyte assemblages differed among storage types (pseudo- $F_{2,\ 13}=2.93,\ P=0.002$ ; Table 4; Fig. 2c), but this was inconsistent across times and storages among storage types (i.e. there was a significant sampling occasion by storage nested within storage type interaction, pseudo- $F_{15,\ 72}=4.28,\ P=0.001$ ), which indicates small-scale spatial and temporal variability. A pair-wise comparison indicated that significant differences existed between WRP and both NWB ( $t=2.31,\ P=0.001$ ) and RWS ( $t=1.60,\ P=0.029$ ) but RWS and NWB did not differ (Table 5). This was in contrast to water quality and sediment grain size, where RWS were different from both other storage types.

The higher percent coverage of *Triglochin procerum* in NWB contributed most to the dissimilarity with WRP, where the species was absent (Table 6). While there was no reliable indicator variable that explained the difference between the RWS and WRP, there was a trend for lower species diversity, percent cover and presence of macrophytes in the WRP, but this was not statistically significant.

There was a significant correlation between the habitat and water quality variables and macrophyte assemblages overall (RELATE Rho = 0.21, P = 0.001). Electrical conductivity, shade cover, alkalinity, and

 $\label{eq:total constraints} \textbf{Table 2} \\ \text{Multivariate results (from PERMANOVA) for differences in physical characteristics across storage types. df = degrees of freedom. Significant results ($\alpha = 0.05$) shown in bold. All data sets were normalised and no transformations were performed.}$ 

| Variable       | Factor                                   | df     | Pseudo-F | P     |
|----------------|--|--------|----------|-------|
| Sediment grain | Storage type                             | 2, 13  | 2.77     | 0.024 |
| size           | Storage (storage type)                   | 15, 15 | 4.43     | 0.001 |
|                | Sampling occasion                        | 1, 15  | 2.17     | 0.104 |
|                | Storage type $\times$ sampling occasion  | 2, 15  | 1.96     | 0.102 |
|                | Storage (storage type) $\times$ sampling | 15, 72 | 1.11     | 0.270 |
|                | occasion                                 |        |          |       |
| Water quality  | Storage type                             | 2, 12  | 3.28     | 0.001 |
|                | Storage (storage type)                   | 15, 14 | 2.49     | 0.001 |
|                | Sampling occasion                        | 1, 14  | 15.18    | 0.001 |
|                | Storage type $\times$ sampling occasion  | 2, 14  | 1.53     | 0.159 |
|                | Storage (storage type) $\times$ sampling | 14, 70 | 7.83     | 0.001 |
|                | occasion                                 |        |          |       |
| All habitat    | Storage type                             | 2, 2   | 4.42     | 0.016 |
| variables      | Sampling occasion                        | 1, 29  | 2.13     | 0.007 |
|                | Storage type $\times$ sampling occasion  | 2, 29  | 0.80     | 0.856 |

the percentage cover of cobble and boulders exhibited the strongest correlation with the macrophyte assemblages (BEST Rho = 0.374, P = 0.010).

#### 3.3. All habitat variables

All environmental and habitat variables (i.e. water quality, sediment grain-size and macrophyte assemblages) were averaged for each site, combined into a single data set and compared among the three water body types (Fig. 2d). This also enabled correlations to be made with the macroinvertebrate assemblages. There were significant differences in habitat between sampling occasions (pseudo- $F_{1, 29} = 2.13$ , P = 0.007; Table 2) as well as among storages (pseudo- $F_{2, 2} = 4.42$ , P = 0.016). Pair-wise comparisons indicated that all storage types were significantly different from each other (Table 3).

#### 3.4. Macroinvertebrates

A total of 91,064 individual macroinvertebrates belonging to 48 taxa (predominantly family) were collected using the two sampling methods across the 18 storages and two sampling occasions (Appendices F and G). The four most dominant taxa found across all the storages were Ceinidae (amphipods; 39%), Daphniidae (water fleas; 25%), Corixidae (water boatmen; 7%) and Ostracoda (seed shrimp; 7%).

## 3.4.1. Kick samples

Kick samples captured a total of 87,356 individuals from 47 families (Appendix F). NWB contained the highest abundance (52%) of macroinvertebrates and Ceinidae was the most abundant family across all water storages, accounting for 39% of all individuals captured. Other dominant taxa captured included Daphniidae (water fleas; 26%), Corixidae (water boatmen; 7%) and Ostracoda (seed shrimp; 7%). Similar numbers of taxa were captured in the first (42,148; 48%) and second (45,208; 52%) sampling rounds.

There were significant differences among storage types (pseudo- $F_{2,2}=4.62$ , P(MC)=0.002; Table 4; Fig. 3a) and between sampling events (pseudo- $F_{1,30}=2.17$ , P=0.013; Table 4). A pair-wise comparison for storage types indicated significant differences between RWS and both WRP (t=2.42, P(MC)=0.007) and NWB (t=2.14, P(MC)=0.023; Table 5). Taxa that were reliable indicators of the difference between RWS and WRP included Daphniidae, Ceinidae, Chironominae, and Physidae, all of which occurred at higher abundances at the WRP, while differences between RWS and NWB were driven by Ceinidae, Ostracoda, Physidae and Chironominae, which were all more abundant at NWB (Table 6).

There was no significant overall correlation between habitat variables and macroinvertebrate assemblages. The environmental variables best correlated with the macroinvertebrate assemblages were pH, the percent coverage of clay/silt cobble, and *Rumex crispus*, although this relationship was not statistically significant (BEST Rho = 0.221, P > 0.05).

#### 3.4.2. Rock scrub samples

Rocks were only sampled on the first occasion from all storages (except for Lake Hamilton because rocks were totally absent). A total of 3708 individuals across 34 families were captured using this method (Appendix G). The dominant taxon was Ceinidae, which accounted for 23% of the total number of individuals sampled. Other abundant taxa included Tateidae (gastropods; 17%), Chironominae (midge larvae; 17%) and Ecnomidae (caddisfly larvae; 16%). There was only one taxon found in rock scrub samples that was not present in kick samples; Aeshnidae (dragonfly nymph).

Macroinvertebrate assemblages differed significantly among storage types (pseudo- $F_{2, 13} = 1.91$ , P = 0.005; Table 4, Fig. 3b) and the covariate (average rock length) was not significant. Pair-wise tests found significant differences between RWS and both WRP (t = 1.59, P = 0.004)

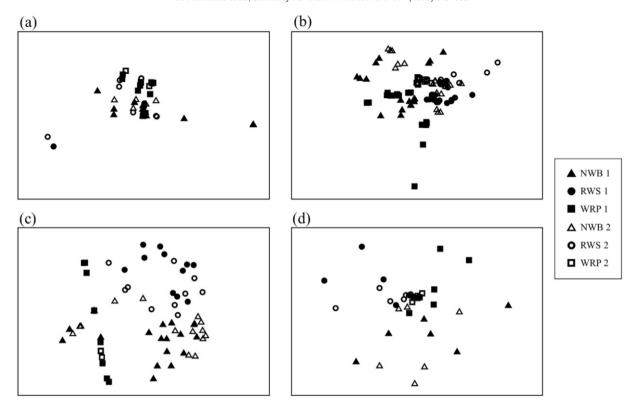


Fig. 2. nMDS ordination plots of the habitat variables: (a) sediment grain size (2D stress = 0.07; n = 108), (b) water quality (2D stress = 0.15; n = 105), (c) macrophyte assemblages (2D stress = 0.16; n = 108), and (d) all habitat variables combined (2D stress = 0.18; n = 35), compared among the three water storages types: natural water bodies (NWB), raw-water storages (RWS) and water reclamation plants (WRP) for each of two sampling times (labelled as 1 or 2). Sediment grain size, water quality and all habitat variables combined were normalised but not transformed, and each MDS plot is based on a Euclidean distance similarity matrix of environmental data. The macrophyte data was square-root transformed and the MDS plot was based on a Bray-Curtis similarity matrix of abundance data with a dummy variable of 1. The ordinations used a maximum of 25 runs.

and NWB (t=1.40, P=0.015; Table 5). Ceinidae and Physidae were consistent indicators of difference between RWS and both other storage types, which was largely consistent with the kick samples (Table 6). There was no significant correlation between macroinvertebrates and the habitat variables. The percent coverage of sand, cobble, *Cyperus* sp. and *Eleocharis sphacelata* were the individual variables best correlated with macroinvertebrates collected from rocks, but this relationship was not statistically significant (BEST Rho=0.401, P>0.05).

#### 4. Discussion

Despite the potential for anthropogenic water storages to act as an effective refuge during disturbance, few studies have assessed and compared macroinvertebrate and macrophyte assemblages in man-made storages with adjacent natural water bodies (Brainwood and Burgin, 2009). This is surprising given that man-made storages can support populations of aquatic biota that can act as a source of colonists following a disturbance, thereby serving as a refuge (Sedell et al., 1990). In making such a comparison, we show that anthropogenic storages can

**Table 3** PERMANOVA pair-wise test results (as probabilities) for each habitat variable showing the probability of significant differences among storage types. Significant results ( $\alpha=0.05$ ) are shown in bold. Monte Carlo probabilities (P(MC)) are reported where the unique permutations available were <100 and are denoted by '\*'.

| Pairwise comparison of storage types | Sediment grain size | Water<br>quality | All<br>habitat |
|--------------------------------------|---------------------|------------------|----------------|
| RWS, WRP                             | 0.129               | 0.001            | *0.007         |
| NWB, RWS                             | 0.324               | 0.004            | *0.016         |
| NWB, WRP                             | 0.003               | 0.312            | *0.011         |

serve as potential refuge habitats, but that the patterns were not as consistent as we had initially predicted. Macroinvertebrate assemblages within water reclamation plants (WRP) were representative of assemblages in surrounding natural water bodies (NWB), whereas macrophyte assemblages were not and, in fact, were absent from half of the WRP sampled. In contrast, raw-water storages (RWS) contained macrophyte assemblages that were representative of NWB, but had dissimilar macroinvertebrate assemblages, and all storage types differed in terms of their available overall habitat. These findings were somewhat contrary to our original hypotheses that RWS would contain assemblages representative of NWB, while WRP would not, because we expected better water quality in RWS and degraded water quality in WRP. The findings of the current study also differ from those of a complementary study of the same storages (Halliday et al., 2015), where no differences among storage types were found for fish, zooplankton or frog assemblages.

## 4.1. Anthropogenic storages as refuge for biota

Despite some of the inconsistencies observed, this study demonstrates that biotic assemblages within anthropogenic storages can be representative of those in surrounding natural water bodies and, thus, could potentially provide a source of colonists following disturbance. We also found that anthropogenic storages consisted of a permanent, stable water source when many of the natural water bodies of equivalent or greater size were dry (Appendix A). However, the two anthropogenic storage types appear to offer refuge differentially for macroinvertebrate and macrophytes, respectively. WRP offered potential refuge habitat for macroinvertebrates during dry conditions, whereas RWS offered potential refuge habitat for macrophytes.

**Table 4**Multivariate results (from PERMANOVA) for differences in assemblages of biota across storage types, df = degrees of freedom. Significant results ( $\alpha = 0.05$ ) shown in bold. Monte Carlo probabilities (P(MC)) are reported where the unique permutations available were <100 and are denoted by \*\*'.

| Variable             | Transformation | Factor  | df     | Pseudo-F | P      |
|----------------------|----------------|---|--------|----------|--------|
| Macrophyte abundance | Square root    | Storage type                                    | 2, 13  | 2.93     | 0.002  |
|                      | •              | Storage (storage type)                          | 15, 15 | 2.99     | 0.001  |
|                      |                | Sampling occasion                               | 1, 15  | 2.48     | 0.047  |
|                      |                | Storage type × sampling occasion                | 2, 15  | 1.20     | 0.289  |
|                      |                | Storage name (storage type) × sampling occasion | 15, 72 | 4.28     | 0.001  |
| Kick samples         | Log(x + 1)     | Storage type                                    | 2, 2   | 4.62     | *0.002 |
| -                    |                | Sampling occasion                               | 1, 30  | 2.16     | 0.013  |
|                      |                | Storage type × sampling occasion                | 2, 30  | 0.64     | 0.916  |
| Rock scrub samples   | Square root    | Storage type                                    | 2, 13  | 1.91     | 0.005  |
|                      | -              | Rock size (covariate)                           | 1, 13  | 1.00     | 0.443  |

The findings for macroinvertebrates in WRP are supported by those of Becerra Jurado et al. (2009), who found macroinvertebrate assemblages in waste-water treatment storages that resembled those in the surrounding landscape, and Brainwood and Burgin (2009), who found that farm dams could be providing refuge for macroinvertebrates during flow disturbances in nearby rivers. These findings are substantive as few previous studies have considered the potential biodiversity value of WRP because they tend to be isolated, particularly in developed landscapes (Chester and Robson, 2013).

In contrast, macroinvertebrate assemblages in RWS were highly variable. Some RWS had assemblages that appeared similar to the other two water body types, whereas others were depauperate and contained very few macroinvertebrates, potentially as a result of differing water quality management. For example, Cobden Service Basin and Plantation Rd. Storage contained the lowest abundances and were the least diverse of the storages sampled. The former was chemically treated with copper sulphate in 2013 to control an algal bloom, and the latter was treated in 2010, while others had not been treated to our knowledge (Burns, pers. comm.). We were unable to identify similar studies in RWS with which to compare with the results of this present study, potentially as anthropogenic storages are often ignored as important habitat (Chester and Robson, 2013), making it difficult to comment on any other factors that may have contributed to the patterns observed here.

For the anthropogenic storages to be an effective refuge for freshwater biota, they must act as a source of colonisation into the broader landscape during optimal times (Robson et al., 2008). None of the storages were directly hydrologically connected with nearby water bodies so the main mechanisms of recolonization would be aerial dispersion for macroinvertebrates and wind or zoochory for macrophytes (Cronk and Fennessy, 2001). While confamilials can vary in their dispersal characteristics (e.g. Leptophlebidae mayflies, Baggiano et al., 2011), Morris (2012) reviewed dispersal techniques of aquatic biota in Victorian wetlands and identified invertebrate taxa which were generally strong flyers compared with weak flyers (Appendix F). The majority of active dispersers were found in the storages sampled here. Of the macrophyte species in this study, waterbirds have been observed dispersing viable seeds of Typha orientalis and Eleocharis sphacelata (both via endozoochory; Appendix E; Morris, 2012). Three Juncus spp. and a Trichlogin sp. are also known to be dispersed by waterbirds (Morris,

**Table 5**PERMANOVA pair-wise test results (as probabilities) for each biotic assemblages showing the probability of significant differences among storage types. Significant results ( $\alpha = 0.05$ ) are shown in bold. Monte Carlo probabilities (P(MC)) are reported where the unique permutations available were < 100 and are denoted by \*\*\*.

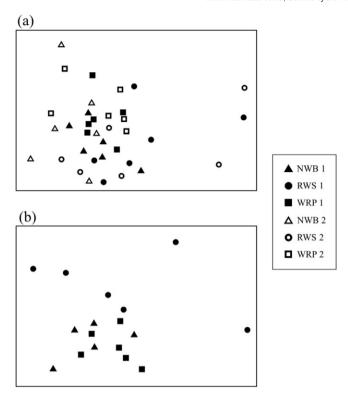
| Pairwise comparison of storage types | Macrophyte   | Kick    | Rock scrub |
|--------------------------------------|--------------|---------|------------|
|                                      | abundance    | samples | samples    |
| RWS, WRP                             | <b>0.029</b> | *0.007  | 0.004      |
| NWB. RWS                             | 0.079        | *0.023  | 0.015      |
| NWB, WRP                             | 0.001        | *0.064  | 0.599      |

2012). Typha orientalis would be the only species found in this study that is adapted for wind-mediated dispersal (Soons, 2006). The relative proximity of the anthropogenic storages to nearby rivers (e.g. Casterton WRP was ~150 m from the Glenelg River) and natural lentic water bodies (e.g. Terang WRP was ~9 km away from Cobrico Swamp) suggests that it would be possible for both aerial adult macroinvertebrates and

Table 6

Results of SIMPER analysis displaying taxa contributing consistently to the observed difference among pairs of significantly different water storage types for the macrophyte and both macroinvertebrate data sets. Taxa are listed in decreasing order of their relative contribution to the total dissimilarity for each pair of water storage types. Average dissimilarity (Av. diss) is each taxon's contribution to the total average dissimilarity. Diss/SD is the ratio of contribution divided by the standard deviation (SD) of those contributions across all pairs of samples (Clarke and Gorley, 2006). Taxa with a Diss/SD value > 1 are considered to be reliable indicators between water storage types (Clarke and Gorley, 2006). Therefore, any taxa with a Diss/SD value < 1 have not been shown. % Contribution shows the contribution for each taxon to the total dissimilarity for each water storage type-pair comparison. The water storage type that was not included in each pair-wise comparison has been left blank. Raw average abundances are shown for each taxon (except for macrophytes which are shown as percent coverage), although analyses were undertaken on square-root or log-transformed data as described in the Methods.

| Method and taxon  | Average abundance for Storage type: |         |      |       |         |              |
|-------------------|-------------------------------------|---------|------|-------|---------|--------------|
|                   | NWB                                 | WRP     | RWS  | Av.   | Diss/SD | %            |
|                   |                                     |         |      | diss  |         | contribution |
| Macrophyte        |                                     |         |      |       |         |              |
| species           |                                     |         |      |       |         |              |
| Triglochin        | 11.2 >                              | 0.0     |      | 28.41 | 1.12    | 29           |
| procerum          |                                     |         |      |       |         |              |
| Kick samples      |                                     |         |      |       |         |              |
| Daphniidae        |                                     | 144.5 > | 1.3  | 7.32  | 1.40    | 12           |
| Ceinidae          |                                     | 113.4 > | 58.1 | 5.09  | 1.11    | 8            |
| Chironominae      |                                     | 37.5 >  | 10.5 | 4.65  | 1.13    | 7            |
| Physidae          |                                     | 22.8 >  | 2.0  | 4.14  | 1.20    | 7            |
| Dytiscidae        |                                     | 11.7 >  | 6.0  | 2.96  | 1.20    | 5            |
| Corixidae         |                                     | 74.9 >  | 65.0 | 2.81  | 1.25    | 4            |
| Leptophlebiidae   |                                     | 0.1 <   | 7.2  | 2.78  | 1.20    | 4            |
| Atyidae           |                                     | 2.9 >   | 0.5  | 2.02  | 1.05    | 3            |
| Ceinidae          | 625.4                               | >       | 58.1 | 5.27  | 1.08    | 8            |
| Ostracoda         | 37.9                                | >       | 1.3  | 4.64  | 1.19    | 7            |
| Physidae          | 49.9                                | >       | 2.0  | 4.42  | 1.46    | 7            |
| Chironominae      | 61.8                                | >       | 10.5 | 3.98  | 1.19    | 6            |
| Coenagrionidae    | 8.0                                 | >       | 1.0  | 2.76  | 1.19    | 4            |
| Leptophlebiidae   | 0.4                                 | <       |      | 2.45  | 1.22    | 4            |
| Dytiscidae        | 3.7                                 | <       |      | 2.18  | 1.26    | 3            |
| Acarina           | 4.6                                 | >       |      | 2.15  | 1.24    | 3            |
| Atyidae           | 4.8                                 | >       | 0.5  | 2.11  | 1.31    | 3            |
| Rock scrub sample | s                                   |         |      |       |         |              |
| Chironominae      |                                     | 60.5 >  | 0.7  | 18.11 | 1.18    | 21           |
| Ceinidae          |                                     | 35.8 >  | 2.4  | 13.34 | 1.07    | 15           |
| Physidae          |                                     | 13.1 >  | 0.1  | 7.57  | 1.07    | 9            |
| Ceinidae          | 76.7                                | >       | 2.4  | 18.02 | 1.67    | 21           |
| Physidae          | 13.1                                | >       | 0.1  | 6.08  | 1.01    | 7            |
| Ecnomidae         | 5.2                                 | >       | 1.4  | 5.79  | 1.03    | 7            |
| Hirudinea         | 5.3                                 | >       | 0.1  | 5.30  | 1.18    | 6            |
| Dytiscidae        | 3.3                                 | >       | 0.0  | 5.25  | 1.62    | 6            |



**Fig. 3.** nMDS ordination plots of the macroinvertebrate assemblages: (a) kick samples (2D stress = 0.21; n = 36), (b) rock scrub samples (2D stress = 0.09; n = 17), compared among the three water storages types: natural water bodies (NWB), raw-water storages (RWS) and water reclamation plants (WRP). 2D stress was high for kick samples thus the MDS plot should be interpreted with caution. Both MDS plots are based on a Bray-Curtis similarity matrix of abundance data. Kick samples were  $\log(x + 1)$ -transformed and the rock scrub samples were square-root transformed. The ordination used a maximum of 25 runs.

macrophyte diaspores to recolonise these systems under future drought scenarios.

## 4.2. Important physical characteristics for biota

A secondary aim of this study was to examine the relationship between biotic assemblages, habitat variables and environmental variables. No habitat variables were consistently associated with diverse macrophyte and macroinvertebrate assemblages. Instead, the variables that tended to be correlated varied among the comparisons made. However, there was a general trend for variables which could be considered indicative of high habitat diversity to be associated with more diverse and abundant biotic assemblages, which is broadly consistent with Chester and Robson (2013).

Macrophyte assemblages were shown to be related to a number of environmental variables measured during this study including electrical conductivity, shading, alkalinity and the percent coverage of cobble and boulders. Electrical conductivity was initially found to be positively correlated with macrophyte coverage, which was unexpected because salinity is widely known to adversely affect freshwater macrophytes (Appendix H). When Lake Cartcarrong was removed as an outlier (4728.8  $\mu S$  cm $^{-1}$ ), the relationship switched to a negative correlation, illustrating the leverage of that single point. Australian macrophytes are generally intolerant of increasing salinity and begin to suffer a range of effects including reduced growth and development, and suppressed reproduction at electrical conductivities above 1500  $\mu S$  cm $^{-1}$  (Hart et al., 1991; Nielsen et al., 2003). Univariate relationships with the percent coverage of cobble and boulders were weak and may have been related to the idiosyncratic nature of substrates within the storages (e.g. WRP

were comprised almost entirely of cobbles but were largely devoid of macrophytes).

There were no clear correlations between macroinvertebrate assemblages and any of the measured habitat variables. Similarly, patterns in macroinvertebrate assemblages were not consistent with patterns in macrophyte coverage. This was somewhat unexpected, given that macrophytes can be very important habitat for macroinvertebrates, tending to support more abundant and more diverse assemblages than unvegetated areas due to differences in habitat (Warfe and Barmuta, 2004) and differences in both direct (Gregg and Rose, 1982) and indirect provisions of food (e.g. epiphytic periphyton; Lamberti and Resh, 1983). Instead, other factors may have driven macroinvertebrate assemblages, such as nutrient concentrations (e.g. Spieles and Mitsch, 2000), which could be incorporated into future study designs.

#### 4.3. The impact of dry conditions and drought

The WRP and the NWB were found to have similar water quality and support similar macroinvertebrate taxa, which was contrary to our initial hypotheses. However, it is important to note the relatively unusual weather conditions that preceded each sampling occasion. The first sampling occasion was undertaken immediately after a very dry summer (Steffen and Hughes, 2013) and most of the NWB were in a drying phase. The second sampling occasion was undertaken during late winter, following somewhat above-average rainfall for the area over the ~4 months between the sampling occasions (390 mm *c.f.* 305 mm on average; www.bom.gov.au), which tended to return the water bodies to more usual wetter conditions. This increased rainfall, and higher water levels, may have contributed to observed differences in habitat and macroinvertebrate assemblages between sampling occasions. However, it did not change the pattern of WRP and NWB having similar macroinvertebrate assemblages, which was consistent across sampling occasions.

The initial dry conditions may have led to degradation of water quality, and therefore sensitive taxa may have already been excluded or lost from the NWB sampled. Thus, this may have meant that NWB were, in fact, in a state of stress rather than representing water quality that is typical of 'average' conditions. Even though the subsequent sampling occasion occurred after a return to wetter conditions, replenishing water levels in the water bodies, it is unlikely that there would have been sufficient time for the biotic assemblages to completely recover from this stress (Bond et al., 2008). In order to assess whether taxa that were sampled from NWB were representative of a wider range of conditions (e.g. wetter periods), we compared the taxa sampled here to those reported in previous studies in the region (Robson and Clay, 2005; Bovill, 2006; Carr, 2011; Dickson, 2015; Appendices I and J). Robson and Clay (2005) did not provide a complete list of taxa, but identified the number of sensitive taxa were characteristic of the seasonal pasture and non-pasture wetlands that they studied. These included one Trichoptera species (Triplectides australis) and three Ephemeroptera species (Leptophlebidae sp.1, Atalophlebidae AV6 sp.1 and sp.2) all of which belong to families identified here, and one species from the family Gripopterygidae (*Leptoperla varia*) which was not observed in this study. Bovill (2006) identified 38 taxa in NWB in the same region, of which 20 were also found in this study (Appendix I). The 16 taxa that were not identified in this study included a range of groups such as Aeshnidae (dragonfly), Baetidae (mayfly), various Hemiptera (true bugs) and Stratiomyidae (true fly). However, an additional 19 taxa were identified in this study that were not found by Bovill (2006), resulting in similar diversity across the two studies.

For macrophytes, we tended to find fewer genera than other similar studies (*c.f.* 11 genera at NWB compared with 13 to 19 in Carr, 2011 and Dickson, 2015; Appendix J). Genera that were not found in this study included several sedges (e.g. *Baumea, Isolepsis*), several floating macrophytes (e.g. *Lemna, Callitriche*) and several weed species (e.g. *Ranunculus, Mentha*) (Appendix J). However, this study identified a

number of submerged macrophytes (e.g. *Elodea*, *Vallisneria*, *Lepilaena* and *Ruppia*) that were not identified in other studies. This suggests that the preceding dry weather conditions may have led to some depletion of macroinvertebrate and macrophytes assemblages within these storages but, based on the limited available information, we appeared to sample taxa that were representative of previous studies.

## 4.4. Generality of findings

Preceding dry conditions made it difficult to find NWB that physically resembled the RWS and WRP, especially those of equivalent size (i.e. NWB were often larger than the anthropogenic storages). This difficulty reinforces the importance of anthropogenic storage as potential refuges for biota given that most of the small NWB were dry during hot, dry conditions while the anthropogenic storages remained wet. Furthermore, we did not detect significant differences in water quality, macrophyte diversity and macroinvertebrate assemblages associated with size between NWB and the anthropogenic storages.

Also, individual management regimes of all the anthropogenic storages were not known prior to sampling. This may have resulted in a choice of storages that had a range of unknown, and potentially variable, impacts on water quality and biotic assemblages. This is particularly likely for the RWS as they generally possessed seemingly-suitable habitat for macroinvertebrates but had extremely variable macroinvertebrate assemblages. It would be advisable for future studies to incorporate any information regarding previous treatment, as it allows storages to be categorised according to their treatment, which could have potentially explained the large variation in the biotic assemblages in RWS.

#### 4.5. Preservation of biodiversity within a landscape

A diverse range of water body types within a catchment is important for the preservation of freshwater biodiversity within the region, as different water body types support different taxa (Williams et al., 2004). Anthropogenic water storages contribute to this diversity of available water bodies, and this study and others illustrate that they can contribute to the biodiversity of macroinvertebrates (e.g. Céréghino et al., 2008; Becerra Jurado et al., 2009; Brainwood and Burgin, 2009) and macrophytes (Linton and Goulder, 2000; Armitage et al., 2003; Goulder, 2008). Thus, the potential contribution of anthropogenic storages should be recognised in conservation and management when considering the preservation of freshwater biodiversity. In order for these storages to act as refuges for macroinvertebrate and macrophytes, their habitat characteristics should, at a minimum, be maintained and habitat complexity maximised, with particular emphasis on diversifying macrophyte assemblages and substrate types in the WRP storages. However, it is also important to note that these storages have important functional purposes (e.g. supply of potable water), so any habitat enhancement practices need to be balanced so that the function of these utilities remains largely unimpeded.

## 5. Conclusion

WRP and RWS supported either macrophyte or macroinvertebrate assemblages that were representative of nearby natural lakes. They also provided a stable, permanent water source when natural systems of equivalent size were dry. Thus, we consider that these anthropogenic water bodies may offer freshwater biota refuge during drought, albeit in different (though complementary) ways. WRP supported macroinvertebrate assemblages that were representative of the surrounding landscape, although co-occurring macrophyte assemblages were depauperate. In contrast, RWS contained macrophyte assemblages representative of the surrounding landscape but had highly variable macroinvertebrate assemblages. Therefore, individual RWS are unlikely to consistently provide reliable refuge for macroinvertebrates, which is

possibly dependent on the water quality management regimes. Storages with a range of substrate types and diverse macrophyte assemblages appeared to support the greatest biodiversity. These findings highlight the importance of anthropogenic storages for the preservation of aquatic biodiversity as other refuges become scarcer under future climate change.

## Acknowledgements

This research was conducted as a part of David Dodemaide's Honours degree. We would like to thank Bryce Halliday, Patrick Pickett and Matthew Linn for their research assistance. Data for biotic assemblage comparisons was generously supplied Lachlan Farrington of Nature Glenelg Trust, Wim Bovill of The University of Melbourne and Geoff Carr of Ecology Australia. Thanks also to Agnes Lautenschlager, Jan Barton, David Mills and Sharon Rowe for advice and support. We also acknowledge the contribution of Wannon Water (26659) and Deakin University for funding to support this project. We would like to thank Ashley Burns, Ian Bail and Julie Rissman from Wannon Water in particular for their support throughout the project and comments on this manuscript. Finally we would like to thank the two anonymous reviewers for their helpful comments and feedback. This research was conducted in accordance with the regulations of the Deakin University Animal Ethics Committee under Permit AEC06.11 and in accordance with the Victorian Department of Primary Industries Fisheries Research Permits RP1026, RP1052 and RP1076.

#### Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.10.333.

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